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Abstract

To date, the knowledge of the helminth communities of Antarctic birds is scarce or fragmented. Knowledge about diseases and parasites is crucial for understanding and managing ecosystems, particularly in isolated areas where host species are more sensitive to new diseases or parasite infections. It has been showed that variations in rate of parasitism may occur between populations of host species. Two major non exclusive hypotheses have been proposed to explain such variability: exposure to parasitism and, perhaps more important, life history strategies of hosts. We studied the helminth community of the Kerguelen Shag *Phalacrocorax verrucosus*, an endemic seabird species of the Kerguelen Archipelago. We provide new data on the helminths infecting this species from partial or complete digestive tracts of two birds. Two nematodes (*Contracaecum rudolphii* s.l. and *Ingliseria cirrohamata*) were found free or attached to the wall of the proventriculus of birds, while the acanthocephalan *Corynosoma* sp. and the cestode species *Tetrabothrius* sp. occurred in the intestine of the shags. The genus *Tetrabothrius* is reported for the first time in Kerguelen Shags and in this area. The analysis of stomach contents from 41 live Kerguelen Shag individuals revealed infection by *Contracaecum* nematodes. The proportion of infected birds differed between colonies, possibly in relation to differential exposure to infected fish hosts.

Keywords: Nematoda, Cestoda, Acanthocephala, Southern Ocean, Cormorant, Seabird.

Introduction

Parasites can affect the population dynamics of their hosts. This may be determinant for isolated populations, and endemic or endangered species (*e.g.* Warner 1968) as a result of less developed immune defences (Lindström et al. 2004). To a large extent, bird and mammal species living in the Antarctic and sub Antarctic regions are endemic. To date, knowledge of the parasite fauna of these definitive hosts is virtually unknown (Barbosa and Palacios 2009), as are the processes of infection in these areas.

The Blue-eyed Shag complex is a group of 13 species exhibiting close morphological and behavioural characters, and which are loosely distributed around the Southern Ocean between roughly 40°S and 70°S (Orta 1992), in more or less complete isolation from one another. The Kerguelen Shag *Phalacrocorax verrucosus* (Cabanis, 1875) (Phalacrocoracidae: Cormorants) is a member of this species complex and is endemic to the Kerguelen Archipelago. This seabird is a marine piscivorous diver which feeds on fish from the Notothenioid sub-order (Cook et al. 2013), a group of benthic fish evolved in the Southern Ocean. Its population is estimated around 7000 breeding pairs (Weimerskirch et al. 1988), although this figure is probably approximate. The helminth fauna of this bird host is not well known. Three species are currently the only recorded parasites in Kerguelen Shags: the nematodes *Ingliseria cirrohamata* (Linstow, 1888) and *Contracaecum rudolphii* s.l. Hartwich, 1964, both first described in the Kerguelen Shag in the late 19th century (Linstow 1888) and confirmed later (Cram 1927; Johnston and Mawson 1945) and one acanthocephalan species (*Corynosoma clavatum* Goss, 1940), described in the mid-20th century (Edmonds 1957). The aim of this work is to contribute to the knowledge of the gastrointestinal helminth community of Kerguelen Shags, and more largely of Antarctic and Subantarctic seabirds, and to discuss potential sources of infection.

Materials and methods

The study was carried out at the Kerguelen Archipelago (49°S, 69°E) during the Austral summer from December 2005 to March 2006. Parasites came from two sources: live bird regurgitations and dead bird carcasses. Forty-one spontaneously regurgitated stomach contents were collected while handling 41 nesting birds upon their return from the sea (contents weighing less than 18g were not kept for analysis). Stomach contents came from the Pointe Suzanne (n=18) and Sourcils Noirs (n=23) shag breeding colonies (colonies 30 km distant from one another, Fig. 1). In addition, the entire digestive tract of a fresh adult Kerguelen Shag found dead at Ratmanoff locality and the stomach (intestine too damaged for analyses) of one immature bird collected dead at Mayès were studied for parasitical examination (Fig. 1). After collection, carcasses and regurgitations were rapidly frozen and conserved at –20°C until examination.

Samples were thawed at the laboratory. All worms were collected from regurgitates while these were sorted for diet analysis (Cook et al. 2013) using a binocular magnifying glass (× 2.5). Contents of digestive tracts were macroscopically examined. All worms collected in regurgitates and digestive tracts were counted, fixed in 10% formalin, and stored in 70% alcohol before identification to genus or species level whenever possible. Parasite identifications were carried out using a list of specific bibliography on helminths of Subantarctic and Antarctic seabirds (*e.g.* Cram 1927; Johnston 1937a,b; Diaz et al. 2009) or Austral seabirds (*e.g.* Goss 1940).

Occurrence and intensity of infection in nematodes were tested using Chi-square test and Mann-Whitney test respectively

The study was approved by the French Ethic Committee of the Institut Polaire Paul-Emile Victor (IPEV) and by the Polar Environment Committee of the Terres Australes et Antarctiques Françaises (TAAF).

Results

During our study, only the nematode *Contracaecum rudolphii* sensus lato (s.l.) was found in the 41 regurgitates of nesting adults. In total, 34.1% of regurgitates contained parasites. Occurrence of infection was significantly different between colonies (Chi-square test: $\chi^2(1) = 4.36$, $p<0.05$) (Table 1). Infection ranged between 0 and 28 nematodes per regurgitate and was also significantly different between colonies (Mann-Whitney test: $U(39) = 129.5$, $Z = –2.02$, $p <0.05$) (Table 1). Four helminth genera (of which two were identifiable to the species level) were found in the digestive tract of the two carcasses of Kerguelen Shags. Two nematode species, *Contracaecum rudolphii* s.l. and *Ingliseria cirrohamata*, were found free or attached to the wall of the proventriculus of birds. Two genera, the acanthocephalan *Corynosoma* sp. and the cestode *Tetrabothrius* sp., occurred in the intestine of the shags. The digestive tract of the adult bird contained 1050 *Tetrabothrius* sp., 101 *Contracaecum rudolphii* s.l., 207 *Ingliseria cirrohamata* and 161 *Corynosoma* sp. The stomach of the fresh immature bird contained 289 *Contracaecum rudolphii* s.l.

Discussion

In all, only four genera of helminths (in which two species) were found in the Kerguelen Shag and more examinations will be necessary to confirm these results and to identify at specific level some of the species found. Despite a modest sample size, the helminth community of the digestive tract of the Kerguelen Shag appears poor compared to that of more northerly and continental Phalacrocoracidae species in which up to 17 species can occur, as in the Great Cormorant *Phalacrocorax carbo* in Europe (*e.g.* Švažas et al. 2011), the Double-crested Cormorant *Phalacrocorax auritus* in North America and the Neotropic Cormorant *Phalacrocorax brasilianus* in South America (*e.g.* Fedynich et al. 1997). The helminth richness in Kerguelen Shags is in accordance with the relatively low number of parasites species (from 3 to 6) found in other Sub-Antarctic and Antarctic piscivorous birds (Fonteneau et al. 2011; Vidal et al. 2012; Diaz et al. 2013). Such latitudinal differences in helminth diversity could result from the global tropic-to-pole diversity gradient (Turner 2004) and isolation of Sub-Antarctic and Antarctic species, leading to lower parasite richness in these regions.

Tetrabothrius sp. was present in the intestine of the adult Kerguelen Shag. This is the first report of this cestode genus in the Kerguelen Shag and in this geographical area. This genus occurs also in two other Phalacrocoracidae birds (the Double-crested Cormorant *Phalacrocorax auritus* and in the Antarctic Shag *Phalacrocorax bransfieldensis*) (Hoberg 1987) and throughout a wide range of Sub-Antarctic and Antarctic bird host species, like penguins (*e.g.* Fonteneau et al. 2011; Vidal et al. 2012) and petrels (*e.g.* Jones 1988). More investigations should be conducted to identify the status of the *Tetrabothrius* species infecting the Kerguelen Shag compared to other *Tetrabothrius* species found in other seabirds.

Our study reports for the second time acanthocephalans of the genus *Corynosoma* from the intestine of the adult Kerguelen Shag. Further investigations should be conducted to identify the status of the *Corynosoma* sp. found here compared to *Corynosoma clavatum* previously described in the Kerguelen Shag (Edmonds 1957).

The Acuariidae nematode *Ingliseria cirrohamata* was first described in the Kerguelen Shag at Kerguelen Archipelago (Linstow 1888; Cram 1927; Johnston & Mawson 1945). Recently, this nematode was re-described with new samples from this type host and locality and in three other bird species (the Imperial Shag *Phalacrocorax albiventer*, the Neotropic Cormorant and the Magellanic Cormorant *Phalacrocorax magellanicus*) from two other geographical localities (Argentina and Chile) (Diaz et al. 2009). In view of our results, infection intensity would seem more important in the Kerguelen Shag (up to 207 specimens in one bird) than in the three other definitive hosts (no more than four specimens per bird) (Diaz et al. 2009). However, no regurgitations of Kerguelen Shags contained *Ingliseria cirrohamata* and only one of the two carcasses was infected. This nematode does not seem a common parasite in this host. Indeed, only two specimens were found in the stomach of one bird during the first parasite exploration in this host species (Linstow 1888; Cram 1927) and later only one specimen was obtained in one of seven examined birds (Johnston and Mawson 1945).

Nematodes of the genus *Contracaecum* (Anisakidae) are known to commonly infect the Phalacrocoracidae family (*e.g.* Fedynich et al. 1997; Abollo et al. 2001; Dezfuli et al. 2002; Garbin et al. 2011). *Contracaecum rudolphii* s.l. was described infecting the Kerguelen Shag (Linstow 1888). It is now known that *Contracaecum rudolphii* s.l. is formed by a complex of several sibling species (*e.g.* Mattiucci et al. 2008; D'Amelio et al. 2012). The *Contracaecum* species found in the Kerguelen Shag during the present study was genetically analysed and identified as a new sibling species differing from those *Contracaecum rudolphii* s.l. known in other birds (Mattiucci et al. 2009). Further studies are needed to determine which morphological characteristics can be used to distinguish this species in the *Contracaecum rudolphii* complex in order to assign a formal name to it. We found this nematode at all sites, which is consistent with a previous study which reported it at "various localities in Kerguelen" (Johnston and Mawson 1945), suggesting it is distributed throughout the archipelago.

Contracaecum nematodes have been reported attached to the wall of the proventriculus of some piscivorous birds. This fastening of nematodes is thought to be an adaptation to counter the abrasive effect of fish bones resulting from digestion (Huizinga 1971; Liu and Edward 1971). Thus, this attachment seems to be effective only towards the end of the digestive period. During the digestive process, most nematodes are normally free and mixed to the stomach content. As regurgitates in the present study come exclusively from birds just returning from fishing areas, we assumed that nematodes were free in the stomach at the moment of regurgitation. Nevertheless, as spontaneous regurgitations represented a fraction of the stomach content, the number of nematodes collected per bird amounted only to a certain percentage of all the nematodes actually present in the whole stomach. *Contracaecum rudolphii* s.l. was found in the stomachs of the two carcasses from the present study, and in all seven birds examined previously by Johnston and Mawson (1945). However, occurrence of this nematode in regurgitations ranged only from 17 to 48%, depending on the colony. Also, *Contracaecum* numbers per regurgitations (from 0 to 28) were low compared to the numbers of nematodes found in the stomachs of the two carcasses (from 101 to 289). Obviously, the regurgitation method does not provide an absolute figure of the parasite load in the bird's stomach, but an estimate of the number per gram of food. The prevalence and intensity at Sourcils Noir colony were higher than at Pointe Suzanne despite the average mass of stomach content being lower at the former, suggesting that infection estimates were not biased by sample size. Even by extrapolating the number of parasites in each sample to the total theoretical mass of food per content, the number of parasites does not approach the number found in the stomach of the carcasses. Several hypotheses can be suggested to explain this discrepancy. First, not all *Contracaecum* are detached from the proventriculus when the bird's stomach is full. Second, the deaths of the two Kerguelen Shags found dead could be related to their high infections in *Contracaecum*. The first description of this nematode genus in Kerguelen Shags reports a bird with only 36 nematodes (Linstow 1888), suggesting that the numbers found in the two carcasses could be indeed higher than normal. Future investigations should focus on testing the efficiency of parasitical study of regurgitations as a non-destructive method to evaluate infection rates of stomach nematodes in piscivorous birds.

The diet of Kerguelen Shags is comprised of nearly 90% of nothotenioid fish species (Table 1), namely *Lepidonotothen mizops*, *Notothenia cyanobrancha* and *Harpagifer* sp. (Cook et al. 2013). In Sub-Antarctic and Antarctic areas, some nototheniid fish, amongst which the three genera *Lepidonotothen*, *Harpagifer* and *Notothenia*, were mentioned as vectors for larval stages of *Contracaecum* nematodes (*e.g.* Laskowski and Zdzitowiecki 2005; Rokicki et al. 2009). The dominance of *Lepidonotothen mizops* at Sourcils Noirs, the colony with the highest infection rates of *Contracaecum* in stomach contents (Table 1), points to a possible determining role of that fish species as a vector of *Contracaecum rudolphii* s.l., even if *Notothenia cyanobrancha* is also known as a possible vector for *Contracaecum* larvae in waters off Kerguelen Archipelago (Johnston and Mawson 1945). Fish of the family Nototheniidae, which are identified vectors for larval stages of ancanthocephalans of the genus *Corynosoma* (see Zdzitowiecki 1986 for review) and for cestodes of the genus *Tetrabothrius* (Hoberg 1987), could also play a similar role in the helminth infection of the Kerguelen Shags and other birds in Sub-Antarctic areas. Little is known about the life cycle of the genus *Ingliseria*. As all the definitive hosts in which this nematode species were described are fish-eating birds (Diaz et al. 2009), fish are also likely vector for *Ingliseria cirrohamata*.

Acknowledgements

The authors are grateful to the French Polar Institute (Institut Paul-Emile Victor - IPEV) and the Terres Australes et Antarctiques Françaises for financial and logistical means in the field. Field work was approved by the institute's ethics committee and was conducted under IPEV research program number 394 (Diving Seabirds, coordinator: Charles-André Bost). Thanks to John Mike Kinsella for his precious assistance in the identification of parasites and to two anonymous referees to their constructive comments and suggestions.

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Table 1. Characteristics of Kerguelen Shag stomach contents: prey and infection rates. The three most abundant prey species (amounting to almost 90% of all prey items) are presented (data from Cook et al. 2013). Occurrence is defined as the occurrence of the fish or parasite in the content relative to the total number of contents. Proportion is defined as the proportion of individuals from any prey species relative to the total number of prey items in all contents. Values are presented as average (± SD) and range.

	Sourcils Noirs	**Pointe Suzanne**
Stomach content mass (g)	59 ± 38 (18–192)	98 ± 43 (24–179)
Harpagifer sp. occurrence (%)	46.1	38.9
Harpagifer sp. proportion (%)	10.0	7.2
Notothenia cyanobrancha occurrence (%)	19.2	94.4
Notothenia cyanobrancha proportion (%)	3.5	74.0
Lepidonotothen mizops occurrence (%)	92.3	22.2
Lepidonotothen mizops proportion (%)	72.6	7.2
Contracaecum rudolphii s.l. occurrence (%)	47.8	16.7
Contracaecum rudolphii s.l. per content	4.2 ± 7.3 (0–28)	0.2 ± 0.5 (0–2)
Contracaecum rudolphii s.l. per gram	0.083 ± 0.182 (0–0.840)	0.003 ± 0.007 (0–0.025)

Fig. 1. Localisation of sampled sites at Kerguelen Islands during the breeding season 2005-2006. Mayès and Ratmanoff correspond to the names of the sites where the two dead shags were found near the shore. Pointe Suzanne and Sourcils Noirs correspond to the two shag breeding colonies where the regurgitated stomach contents were sampled.